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Research Article

# COMPARATIVE STUDY IN ANTIOXIDANT ACTIVITIES OF THE DIFFERENT RIPENESS STAGES OF *ARTOCARPUS HETEROPHYLLUS* LAM. FRUIT

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**ABSTRACT**

The ethyl acetate extracts obtained from under-ripe (Young Stage), mature (Mature Stage) and ripe fruit (Ripe Stage) fruit pulp of *Artocarpus heterophyllus* Lam. were evaluated for their Total Phenolic Content, Total Flavonoid Content and antioxidant property. Total Moisture Content and the Total Ash Content of pulp were determined by subjecting to heat treatment. Total Phenolic Content was evaluated using Folin-Ciocalteu method and Total Flavonoid Content by Aluminium Chloride Colorimetric Assay. Antioxidant activity was determined by DPPH Radical Scavenging, ABTS Radical Scavenging and FRAP Assays. The highest moisture content varies as Young Stage>Mature Stage>Ripe Stage in 84.71% to 70.38% range and Total Ash Content of the Ripe Stage pulp was the highest (6.86%) and the least was observed for the Young Stage with a value of 5.40%. For the Total Phenolic Content, crude extracts isolated from Mature Stage showed highest value (434.04 mg GAE/g) and Total Flavonoid Content was highest in crude extract of the Young Stage (446.79 mg QE/g). Ripe Stage Crude extract gave lowest value for both Total Phenolic Content and Total Flavonoid Content. For DPPH Radical Scavenging, ABTS Radical Scavenging and FRAP Assays, highest activity was reported by crude extract of Young Stage followed by crude extract of Mature Stage and least activity was given by crude extract of Ripe Stage. A correlation between Total Phenolic Content and Total Flavonoid Content with antioxidant activity was noticeable. A declination of the antioxidant activity was observed as the fruit reaches its maturity.

**KEYWORDS:** Jackfruit, *Artocarpus heterophyllus* Lam., Total Phenolic Content, Total Flavonoid Content, Antioxidant activity.

## INTRODUCTION

The *Artocarpus heterophyllus* Lam. or commonly known as the Jackfruit tree is a frequently found tree in Asian countries including Sri Lanka, India, Thailand, Malaysia, and Bangladesh. This evergreen tree which could grows up to about 28 – 80 feet in height has a straight stem and thick bark with a greenish to blackish shade of color, that exudes milky latex upon incision. Branches could spring from the very low to top most part of the tree and carry broad oval shaped thick green colored leaves.[1]

The tree is well known for its timber that is durable and considered to have anti-termite property. Jackfruit tree's wood has an orange yellowish color and even at ancient times, a dye produced from this wood chips has been used to color the robes of Buddhist monks. Parts of the tree such as bark, roots, fruit, seed and leaves have medicinal properties and are used in indigenous medicinal treatments. It is reported that these parts have antioxidant, anti-inflammatory, antibacterial, antifungal, hypoglycemic and wound healing effects too.[2] Research has demonstrated that Jackfruit can contribute to the reduce of cardiovascular and cancer dieses and due to that has gain a considerable interest among nutrient and food scientists.[1,3] Jackfruit is enriched with vitamins (Vitamin A, Vitamin C, thiamin, riboflavin), minerals, proteins and carbohydrates.[1,2] The fruit also known to have phytonutrients such as lignans, isoflavones, saponins and especially carotenoids which is important in prevention of cancer.[4] Jackfruit is used in development of many value added products and bulbs of ripe fruit can be found as canned fruit items in markets.

The tree is most popular because of its high availability of fruits and is also known as the 'Rice Tree' in Sri Lankan culture. The tree which doesn't have intense nutritional requirements or maintenance, is habitually grow in home gardens and frequent in rural areas of Sri Lanka as a food source. Jackfruit is a seasonal fruit and usually the peak of

harvesting reaches during May/July months. The fruit which takes months to reach the fully riped stage can be distinctly identified as two ecological varieties. One of this types has a soft and spongy pulp and the other type has a firm pulp when the fruit is ripe.[1] In Sri Lanka, Jackfruit is categorized into three stages depending on its maturing or ripeness and in Sinhalese these are known as *'Polos'* (Young stage), *'Kos'* (Mature Stage) and *'Waraka'* (Ripe Stage). Young Stage and Mature Stage fruits are consumed as cooked food items and Ripe Stage is a delicious fruit item that doesn't required pre preparations.

It is reported that certain characteristics of the ripe jackfruit such as starch content, moisture content, pH, sugar and carotenoid content is influenced by both the variety of the Jackfruit and the place too.[5] A study conducted in Sri Lanka evaluating the carotenoid content in jackfruits that were collected from two areas has proven to have carotenoids in different content as well as in composition.[6] In a separate investigation that was conducted to determine the various activities of under ripe, ripe and over ripe pawpaw fruit suggests that factors such as phenolic content, flavonoid content, reducing potential and antioxidant activity is affected by the fruit's ripeness stage.[7] Many research has conducted to evaluate the antioxidant properties of ripe jackfruits peel, pulp and even seeds.[8,9] Here, objectives of this study was to determine the antioxidant activity of the edible pulp part of the Jackfruit at the three maturity stages (Young Stage, Mature Stage and Ripe Stage) and to conduct a comparative study in the activity with the development of the fruit.

## MARETIALS AND METHOD

### Sample collection and extraction

Fruits were identified according to their maturity stages (Young Stage, Mature Stage and Ripe Stage) and separately cut down from trees available in Kegalle District and the edible pulp belongs to the Young Stage, Mature Stage and Ripe Stage was isolated from the individual fruits. These pulp parts were cut in to small pieces and 500g of these pieces were transferred to labeled large conical flasks. Ethyl acetate (500mL) was used as the solvent. The flasks were covered with aluminium foil and kept on an orbital shaker for a day. The ethyl acetate was filtered and rotary evaporated to isolate the crude extracts. Further solvent evaporation and solidification of the collected extracts were achieved under gentle nitrogen stream. The extracts obtained were labeled as Young Stage Crude, Mature Stage Crude and Ripe Stage Crude (YSC, MSC and RSC) and were stored in refrigerator.

### Determination of total moisture content (MC)

A portion of 5.0g of freshly cut pulp portions isolated from Young Stage fruits, was transferred in to an initially weighed watch glass and was dried in a pre-heated oven at 105˚C (± 2 ℃) for 2 hours. The weight was measured after it reaches a constant value. The same procedure was followed to determine the Moisture Content in Mature Stage and Ripe Stage pulp too. Bellow equation (Eq. 1) was used to calculate the moisture content (to expressed as a percentage).

$$MC\ (\%) = \left[\frac{Wi-Wf}{Wi-Wa}\right] \times 100\% \text{(Eq. 1)}$$

Where, Wi - Initial weight of sample + dish, Wf – Final weight of sample + dish and Wa – Weight of the dish.

### Determination of total ash content (AC)

A portion of 5.0g of freshly cut pulp pieces isolated from Young Stage fruits, was dried in an oven (80 ℃) for two hours and then transferred in to an initially weighed porcelain crucible and was heated in a muffle furnace at 550˚C (± 2℃) for 6 hours.[10] The final weight of the sample was taken after the test sample reached to room temperature in a desiccator. The same procedure was followed to determine the ash content in Mature Stage and Ripe Stage pulp. Total ash content was calculated according to the equation 1.

### Determination of total phenolic content (TPC)

The total soluble phenols present in the samples were determined by using Folin-Ciocalteu reagent.[11] The extract sample to be tested (0.5mL) was mixed with 0.5mL of Folin Ciocalteu reagent (1: 1). The sample was allowed to stand for 5 minutes at room temperature and then 0.5mL of sodium carbonate (6% w/v) was added. After adding 3mL of distilled water, the mixture was kept in dark for 60 minutes at room temperature. The absorbance of the resulting solution was observed at 765nm against a blank sample that was prepared in the same way by replacing the extract with distilled water. A series of gallic acid concentrations were used to construct the standard curve ($R^2$=0.97) and was used to measure the phenolic content. The Total Phenolic Content was express as mg GAE/g of weight of the extract.

### Determination of total flavonoid content (TFC)

The flavonoids present in the extract samples (YSC, MSC and RSC) were determined by using aluminium chloride colorimetric assay.[11]A series of Quercetine was used to construct the calibration curve. The absorbance of the test samples were measured at 510nm. The Total Flavonoid Content of the evaluated samples was expressed as mg QE/g of weight of extracts.

**Determination of radical scavenging activity by DPPH assay**

The DPPH radical scavenging activities of YSC, MSC and RSC extracts were determined by following the method reported by Kuganesan et al.,[11] with slight changes. BHT was used as the positive reference. The DPPH radical scavenging ability of the test samples were calculated by using following equation:

$$\text{Scavenging Activity (\%)} = \left[1 - \frac{A_s}{A_o}\right] \times 100\% \text{ (Eq. 2)}$$

Where $A_0$ is the absorbance of the control and as is the absorbance in the presence of extracts or positive standard. The results were plotted as the percentage of scavenging activity against concentration of the sample.

**Determination of radical scavenging activity by ABTS assay**

The method reported by Kuganesan et al., was followed to determine the ABTS•+ radical scavenging activity of the YSC, MSC and RSC extracts.[11] BHT was used as the positive reference for the experiment and absorbance was measured at 734nm after 6 minutes of incubation time. Scavenging activity of ABTS•+ was calculated using equation 2. The percentage of scavenging activity was plotted against the concentration of extracts.

**Ferric reducing power Assay (FRAP Assay)**

A volume of 0.25mL of different concentrations of extracts/standard was mixed with 1.00mL of phosphate buffer (pH 6.6) and 1.00mL of Potassium ferricyanide (1% W/V) and the resultant mixture was incubated at 50˚C for 20 minutes. After rapid cooling of the test sample tricholoro acetic acid (10% w/v) and afterward 1% Ferric chloride was added. The mixture was allowed to stand for 20 minutes and absorbance was measured at 700nm.[11]

Note– In every assay, the tests were triplicated and an average was taken as the final data point or a reading. Data reported as mean ± SD. One way ANOVA was used to determine the difference between means, and p≤ 0.05 was considered to be statistically significant. This statistical analysis was carried out using Microsoft Excel.

**RSULTS AND DISCUSSION**

**Total moisture content and total ash content**

At the end of solvent evaporation, the solid weight of the Young Stage Crude, Mature Stage Crude and Ripe Stage Crude extracts were 1.25g, 0.89g and 1.14g respectively. Moisture content in fruits is very important especially in food analysis as it can be a direct reason for microbial growth in food items. The inorganic residue that remains after removal of water and organic matter in fruits and vegetables are considered as the ash content and give an indication of mineral content. According to data obtained Young Stage fruit pulp had the highest moisture content and the Ripe Stage showed the lowest moisture content with values of 84.7 % and 70.38% respectively. The Ash Content of the Ripe Stage pulp was the highest (6.86%) followed by the Mature Stage and the least was observed for the Young Stage with a value of 5.40%.

The Total Moisture Content of the edible pulp portion showed a decline in the percentage, when going from Young Stage to Ripe Stage, where Ripe Stage pulp had the lowest amount of moisture content compared with the other two stages. These obtained percentages ranges are well in agreement with the reported literature. Total Ash Content of the three stages demonstrated an increment from Young stage to ripe stage, which can be attributing to the accumulation of minerals as the fruit grows to maturity. A study on the various inorganic elements in this ash content would definitely be helpful to determine the nutritional importance of the each and every stage. The percentages of Moisture Content and Ash Content of the three stages of Jackfruit pulp is summarized in the table 1.

**Table 1 Total Moisture Content and the Total Ash Content of Young, Mature and Ripe Jackfruit pulp**

| Sample | Moisture content / % | Ash Content / % |
|---|---|---|
| Young Stage | 84.71 ± 0.76[a] | 5.40 ± 0.13[a] |
| Mature Stage | 76.50 ± 0.26[b] | 6.41 ± 0.15[b, c] |
| Ripe Stage | 70.38 ± 0.20 [c] | 6.86 ± 0.08[c] |

Mean followed by the different letter in the same column differs significantly from each other (p≤ 0.05).

**Total phenolic content and total flavonoid content**

Phenolic compounds found in plants have ability to act as reducing agents and to scavenge free radicals by hydrogen donations. Flavonoids are another group of phytochemical that are mainly responsible for the plethora of colors observed in fruits and vegetables, and contain higher antioxidant activity. Using the Gallic acid standards plot ($R^2$ = 0.97), TPC of Jackfruit extracts were determined. The highest TPC was observed in MSC extract (434.04 ± 7.38 mg GAE/g) while lowest value 52.08 ± 7.03 mg GAE/g was reported for RSC extract.

The TFC values were determined using the plot generated by the Quercetin standards and the highest TFC was detected in YSC extract and lowest in RSC extract with 446.79 ± 3.83mg QE/g and 27.56 ± 1.41mg QE/g respectively. The TFC of the ethyl acetate extract exhibited a distinct decline going from Young Stage (446.79 ± 3.83mg QE/g) to Mature Stage and Ripe Stage (27.56 ± 1.41mg QE/g). The decline in TPC and TFC as the fruit reaches its maturity is also observed in the Pawpaw fruit analysis for its antioxidant activity. A summary of the TPC and TFC is given in the table 2.

**Table 2 Total Phenolic Content and Total Flavonoid Content of YSC, MSC and RSC extract**

| Sample | TPC / (mg GAE /g of extract) | TFC / (mg QE/g of extract) |
|---|---|---|
| Young Stage | 289.54 ± 4.54[a] | 446.79 ± 3.83[a] |
| Mature Stage | 434.04 ± 7.38[b] | 74.63 ± 3.29[b] |
| Ripe Stage | 52.08 ± 7.03[c] | 27.56 ± 1.41[c] |

GAE- Gallic acid equivalent, QE- Quercetin equivalent. Mean followed by different letter in the same column differs significantly from each other ($p \leq 0.05$).

**Figures**

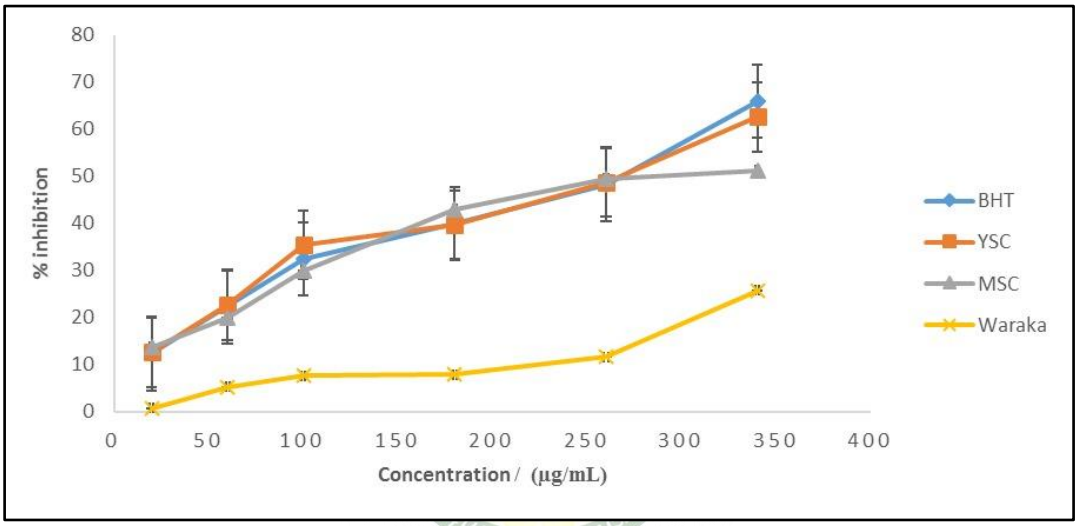


**Figure 1: DPPH radical assay - Percentage Inhibition vs the concentration of YSC, MSC and RSC extracts**

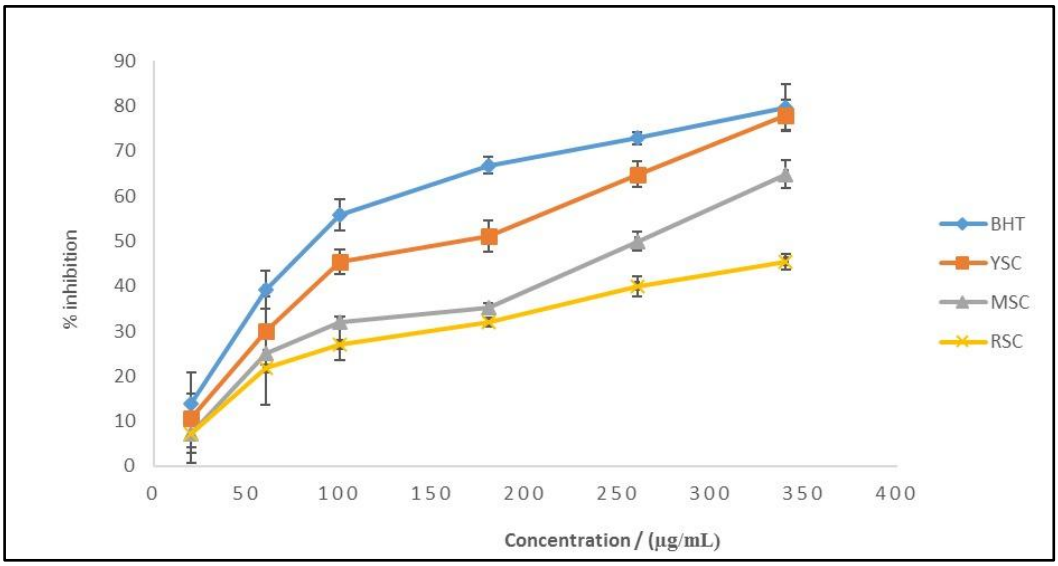


**Figure 2: ABST radical scavenging assay - Percentage Inhibition vs the concentration of YSC, MSC and RSC extracts**

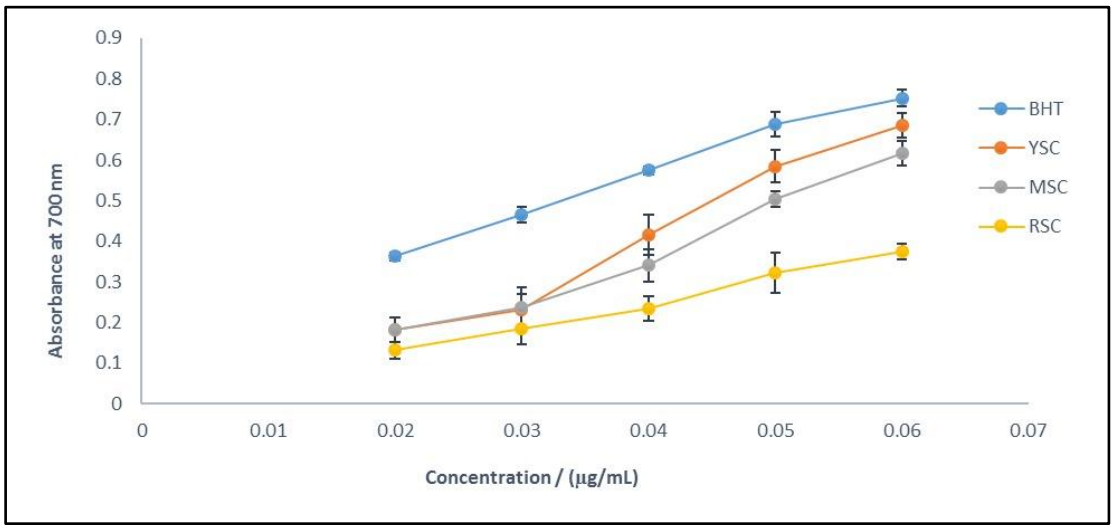


**Figure 3: The absorbance vs the concentration of YSC. MSC and RSC for FRAP Assay**

**DPPH radical scavenging activity assay**

The1,1-Diphenyl-2-picrylhydrazyl abbreviated as the DPPH is very popular assay that is used for the study of antioxidant abilities of natural product extracts. DPPH is a stable free radical which on accepting hydrogen from a corresponding donor loses the characteristic deep purple color. The DPPH free radical scavenging activity was determined for all three extracts.

The $IC_{50}$ values of the positive reference and the extracts indicate that, BHT has the lowest value with 253.15 $\pm$ 2.17$\mu$g/mL. The YSC extract has an $IC_{50}$ value of 255.36 $\pm$ 1.53$\mu$g/mL and MSC extract has a $IC_{50}$ value of 303.06 $\pm$1.96$\mu$g/mL. The highest was observed for RSC extract with a $IC_{50}$ value of 685.73 $\pm$ 2.64$\mu$g/mL. The inhibition percentages of the extracts versus the concentrations are graphically presented in figure 1.

**ABTS scavenging activity assay**

The peroxidase substrate 2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulphonic acid) has the capability of forming a stable radical upon donating an electron and the radical is expressed as ABTS•+. The assay is based upon the ABTS oxidized by persulfate to form green intense color radical action ABTS•+ which has a characteristic absorbance that can be monitored using spectroscopic methods.

The inhibition percentage of the produced ABTS•+ radicals of the standard BHT and extracts were plotted against the concentrations and the generated graph is shown in figure 2. The $IC_{50}$ values of BHT, YSC, MSC and RSC extracts were 99.89 $\pm$ 1.77µg/mL, 190.36 $\pm$ 2.78µg/mL, 280.70 $\pm$ 2.31 µg/mL and 360.06$\pm$ 4.76 µg/mL respectively.

**Ferric ion reducing power (FRAP) assay**

Ferric Reducing power assay (FRAP) was used to measure the direct electron donating ability of extracts. The result visualized by measuring absorbance of blue–green color complex formed at 700nm. The reducing ability of the Young Stage Crude, Mature Stage Crude and Ripe Stage crude were compared with positive standard BHT. Absorbance of the samples at 700nm was plotted against the relevant concentrations to obtain a graphical representation for a clear comparison, which is presented in figure 3.

The highest stabilizing ability was observed for the positive standard used (BHT). In here also we can observe that, lowest is reported to RSC where YSC has a higher stabilizing ability compared to the positive standard used.

**CONCLUSION**

The Total Moisture Content of the edible pulp portion showed a decline in the percentage, when going from Young Stage to Ripe Stage and Total Ash Content of the three stages demonstrated an increment from Young stage to ripe stage. The TFC was highest in YSC and TPC was highest in MSC. The antioxidant activity of ethyl acetate extract obtained from pulp of Jackfruit's vary as YSC>MSC>RSC for DPPH radical scavenging assay, ABTS radical scavenging and FRAP assays. This variation of the antioxidant potential could be explained by the TPC and TFC of the extracts, which considered as main responsible phytochemicals giving antioxidant property.

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